

27

The invention claimed is

1. A process for conjugating a bacterial saccharide and reducing the sizing effect on bacterial saccharide comprising the steps of

a) reacting the bacterial saccharide with 0.001-0.7 molar equivalents of periodate to form an activated bacterial saccharide,

b) mixing the activated bacterial saccharide with a carrier protein;

c) reacting the activated bacterial saccharide and the carrier protein with a reducing agent to form a conjugate;

wherein step a) occurs in a buffer which does not contain an amine group, and the buffer has a concentration between 1-100 mM and wherein the bacterial saccharide is *S.pneumoniae* capsular saccharide 23F.

2. The process of claim 1 wherein the buffer is selected from the group consisting of phosphate buffer, borate buffer, acetate buffer, carbonate buffer and citrate buffer.

3. The process of claim 1 wherein the pH in step a) is pH 3.5-8.0.

4. The process of claim 1 wherein the average molecular weight of the bacterial saccharide is between 1-1100 kDa after step a).

28

5. The process of claim 1 wherein the average molecular weight of the 23F saccharide is between 100-470 kDa after step a).

6. The process of claim 1 wherein the carrier protein is selected from the group consisting of tetanus toxoid, fragment C of tetanus toxoid, diphtheria toxoid, CRM197, Pneumolysin, protein D, PhtD, PhtDE and N19.

7. The process of claim 1 wherein the reducing agent comprises sodium cyanoborohydride or sodium triacetoxymethylborohydride.

8. The process of claim 1 comprising a further step e) of purifying the conjugate.

9. The process of claim 1 containing a further step of mixing the conjugate with further antigens.

10. The process of claim 9 wherein the further antigens comprise one or more *S.pneumoniae* proteins selected from the group consisting of the Poly Histidine Triad family (PhtX), Choline Binding Protein family (CbpX), CbpX truncates, LytX family, LytX truncates, CbpX truncate-LytX truncate chimeric proteins (or fusions), pneumolysin (Ply), PspA, PsaA, Sp128, Sp101, Sp130, Sp125 and Sp133.

11. The process of claim 1 wherein the conjugate is mixed with an adjuvant or a pharmaceutically acceptable excipient.

\* \* \* \* \*